Attorney's Docket No.: 19170-002US1/FR03/03205 US

Applicant: Joanne Tran-Guyon et al.

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Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1-22 (Canceled)

23. (New) A phthalein of general formula (I):

wherein R1, R2, R3, R4 and R5, which are identical to or different from one another, are selected from the group consisting of hydrogen, hydroxyl, halogen, acetyl, amino, phosphate, nitro, sulfonate, carboxyl, alkylcarboxyl having from 2 to 30 carbon atoms, alkyl having from 1 to 30 carbon atoms, cycloalkyl having from 3 to 12 carbon atoms, alkyloxy having from 1 to 30 carbon atoms, haloalkyl having from 1 to 30 carbon atoms, hydroxyalkyl having from 1 to 30 carbon atoms, alkyl ester having from 2 to 40 carbon atoms, nitroalkyl having from 1 to 30 carbon atoms, carboxyalkyl having from 2 to 30 carbon atoms, aminoalkyl having from 1 to 30 carbon atoms, sulfoalkyl having from 1 to 30 carbon atoms, sulfoalkyl having from 1 to 30 carbon atoms, aryl, aryloxy, arylalkyl, haloaryl, aryl ester, succinimidyl ester, isothiocyanate, maleimide, iodoacetamide, haloacetamide, chlorosulfonic, purine or pyrimidine bases, monosaccharides, preferably hexoses or pentoses, oligosides and polyosides, polypeptides, proteins and phospholipids.

R3 and R5 are not hydrogen when R1 is a group -CH₂-CH₂-COOH, R2 is a hydroxyl group and R4 is a group -COOH,

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these phthaleins containing no more than 1% by weight of residual impurities.

24. (New) The phthalein as claimed in claim 23 containing no more than 0.5% by weight of residual impurities.

- 25. (New) The phthalein as claimed in claim 24 containing no more than 0.2% by weight of residual impurities.
 - 26. (New) The phthalein as claimed in claim 23 consisting of fluorescein.
- 27. (New) A method for preparing phthaleins, wherein the residual impurities have been removed, having the general formula (I):

wherein R1, R2, R3, R4 and R5, which are identical to or different from one another, are selected from the group consisting of hydrogen, hydroxyl, halogen, acetyl, amino, phosphate, nitro, sulfonate, carboxyl, alkylcarboxyl having from 2 to 30 carbon atoms, alkyl having from 1 to 30 carbon atoms, cycloalkyl having from 3 to 12 carbon atoms, alkyloxy having from 1 to 30 carbon atoms, haloalkyl having from 1 to 30 carbon atoms, hydroxyalkyl having from 1 to 30 carbon atoms, alkyl ester having from 2 to 40 carbon atoms, nitroalkyl having from 1 to 30 carbon atoms, carboxyalkyl having from 2 to 30 carbon atoms, aminoalkyl having from 1 to 30 carbon atoms, sulfoalkyl having from 1 to 30 carbon atoms, sulfoalkyl having from 1 to 30 carbon atoms, aryl, aryloxy, arylalkyl, haloaryl,

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aryl ester, succinimidyl ester, isothiocyanate, maleimide, iodoacetamide, haloacetamide, chlorosulfonic, purine or pyrimidine bases, monosaccharides, preferably hexoses or pentoses, oligosides and polyosides, polypeptides, proteins and phospholipids,

R3 and R5 are not hydrogen when R1 is a group -CH₂-CH₂-COOH, R2 is a hydroxyl group and R4 is a group -COOH,

wherein a phthalic anhydride derivative of formula (II) is condensed with a phenol or naphthol compound of formula (III)

in which R1, R2, R3, R4 and R5 have the same meanings as above, the condensation being carried out in a solvent consisting of an organic acid ester.

- 28. (New) The method as claimed in claim 27, wherein the compound of formula (III) is selected from the group consisting of resorcinol, orcinol, naphthol, pyrogallol, alkylaminophenol and arylaminophenol.
- 29. (New) The method as claimed in claim 27, wherein the solvent is an organic acid ester of formula (IV)

$$R_6$$
-COOR₇ (IV)

wherein R₆ is selected from the group consisting of hydrogen, alkyl having from 1 to 30 carbon atoms, cycloalkyl having from 3 to 12 carbon atoms, haloalkyl having from 1 to 30 carbon atoms, hydroxyalkyl having from 1 to 30 carbon atoms, nitroalkyl having from 1 to 30

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carbon atoms, aryl, aryloxy, alkylaryl, arylalkyl, substituted arylalkyl, haloaryl, aryl ester, alkyl ester having from 2 to 40 carbon atoms, and alkyloxy having from 1 to 30 carbon atoms, R₇ being selected from the group consisting of alkyl having from 1 to 30 carbon atoms, cycloalkyl having from 3 to 12 carbon atoms, haloalkyl having from 1 to 30 carbon atoms, hydroxyalkyl having from 1 to 30 carbon atoms, nitroalkyl having from 1 to 30 carbon atoms, aryl, aryloxy, alkylaryl, arylalkyl, substituted arylalkyl, haloaryl, aryl ester, alkyl ester having from 2 to 40 carbon atoms, or alkyloxy having from 1 to 30 carbon atoms.

- 30. (New) The method as claimed in claim 27, wherein the organic acid ester is selected from the group consisting of methyl, ethyl, propyl or butyl benzoate, methyl, ethyl, propyl or butyl heptanoate, methyl, ethyl, propyl or butyl octanoate, methyl, ethyl, propyl or butyl laurate, methyl, ethyl, propyl or butyl myristate or methyl, ethyl, propyl or butyl palmitate, and mixtures thereof.
- 31. (New) The method as claimed in claim 27, wherein the condensation reaction is carried out at between 150°C and 250°C, optionally under pressure.
- 32. (New) The method as claimed in claim 27, wherein the reaction is carried out in the presence of a catalyst selected from the group consisting of Lewis acids, such as ZnCl₂ or AlCl₃, Brönsted acids such as H₂SO₄ or polyphosphoric acid.
- 33. (New) The method as claimed in claim 32, wherein the catalyst is an alkali metal hydrogen sulfate.
- 34. (New) The method as claimed in claim 33, wherein the catalyst is potassium hydrogen sulfate (KHSO₄) or sodium hydrogen sulfate (NaHSO₄).

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35. (New) A method for acidifying the product resulting from the condensation of a phthalic anhydride derivative of formula (II) with a phenol or naphthol compound of formula (III), the formulae (II) and (III) being those of claim 27, wherein the reaction is carried out in an anhydrous organic medium, by the addition of a strong acid or one of its precursors, selected from the group consisting of sulfuric acid, hydrochloric acid, hydrobromic acid, hydrofluoric acid, hydriodic acid, polyphosphoric acid, pyrophosphate (P₂O₅), and mixtures thereof, the acidification being carried out until the phthalein crystals resulting from the condensation are converted to phthalein crystals having a different structure.

- 36. (New) The method as claimed in claim 35, wherein the condensation product is the product obtained by the method as claimed in claim 27.
- 37. (New) The method as claimed in claim 35, comprising a step consisting in washing the product obtained after acidification, said washing step being carried out with a washing solution selected from the group consisting of water, alcohols, ketones, ethers and halogenated solvents, pure or as a mixture, until the crystals are reconverted to the structure that they had before the acidification reaction.
- 38. (New) A method for preparing a fluorescein having a purity such that its content of each of the by-products of the reaction is less than or equal to 0.2%, the sum of the contents of each of these by-products being less than or equal to 0.5%, said method comprising the following successive steps:

condensing phthalic anhydride with resorcinol, in a solvent consisting of an ester of an aliphatic or aromatic organic acid, in the presence of a catalyst selected from the group consisting of Lewis acids or Brönsted acids,

suspending the red-colored crystals obtained in the preceding step in an anhydrous solvent selected from the group consisting of alcohols such as absolute ethanol, ketones such as acetone, ethers, halogenated solvents, or mixtures thereof,

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acidifying the suspension thus obtained by the addition of a strong acid or one of its precursors, selected from the group consisting of sulfuric acid, hydrochloric acid, hydrobromic acid, hydrofluoric acid, hydriodic acid, polyphosphoric acid, pyrophosphate (P₂O₅), and mixtures thereof, until the red-colored crystals are converted to yellow-colored crystals exhibiting the X-ray diffraction analysis of figure 2,

washing the crystals obtained with a washing solution selected from the group consisting of water, alcohols, ketones, ethers and halogenated solvents, pure or as a mixture, this washing being continued until the yellow-colored crystals are reconverted to red-colored crystals.

- 39. (New) The method for preparing a fluorescein as claimed in claim 38, having a purity such that its content of each of the by-products of the reaction is less than or equal to 0.1%.
- 40. (New) The method for preparing a fluorescein as claimed in claim 38, wherein the solvent used in the condensation reaction is the ethyl or methyl benzoate or ethyl or methyl palmitate.
- 41. (New) The method for preparing a fluorescein as claimed in claim 37, wherein the catalyst used for the condensation reaction is an alkali metal hydrogen sulfate.
- 42. (New) The method for preparing a fluorescein as claimed in claim 40, the catalyst is potassium hydrogen sulfate or sodium hydrogen sulfate.
- 43. (New) The method as claimed in claim 37, wherein the acidification is carried out by sparging gaseous hydrochloric acid into the phthalein suspension or by the action, on this phthalein, of hydrochloric acid in solution in the anhydrous organic solvent, preferably an alcohol, a ketone, an ether or a halogenated solvent, used alone or as a mixture, even more preferably isopropanol, absolute ethanol or acetone, pure or as a mixture.

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44. (New) A yellow-colored fluorescein crystal having the X-ray diffraction analysis of figure 2.

- 45. (New) A yellow-colored 4',5'-dimethylfluorescein crystal having the X-ray diffraction analysis of figure 4.
- 46. (New) A reddish-brown- or mahogany-colored 4',5'-dihydroxyfluorescein crystal having the X-ray diffraction analysis of figure 6.
 - 47. (New) A phthalein obtained by means of the method as claimed in claim 27.
 - 48. (New) A fluorescein obtained by means of a method as claimed in claim 27.
- 49. (New) A 4',5'-dimethylfluorescein obtained by means of a method as claimed in claim 27.
- 50. (New) A 4',5'-dihydroxyfluorescein obtained by means of a method as claimed in claim 27.
- 51. (New) Pharmaceutical composition for diagnosis, especially for medical imaging comprising the fluorescein as claimed in claim 26.
- 52. (New) Pharmaceutical composition for diagnosis, especially for medical imaging comprising the fluorescein obtained according to the method of claim 27.
- 53. (New) Labeling composition for biotechnological applications comprising the fluorescein as claimed in claim 26.

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54. (New) Labeling composition for biotechnological applications comprising the fluorescein obtained according to the method of claim 27.